Abstract

Cancer is a major health problem, worldwide, and is the second-most frequent cause of death (www.uzis.cz). Research is urgently necessary to reduce cancer incidence and the costs associated with cancer management, to develop more efficient and effective risk prediction strategies and personalised patient treatment. Germinal mutations in genes that predispose individuals to hereditary cancer syndromes are clinically important and can be used to classify carriers being at high risk of cancer development. Identification of these mutations can influence the prognosis and treatment of probands and can be used to include their family members into high-risk groups with corresponding preventive strategies.

This study is focused on the currently underestimated description of newly identified genetic factors among the Czech population that predispose individuals to develop pancreatic ductal adenocarcinoma (PDAC). In 2017, the incidence of PDAC in the Czech Republic was 21 cases per 100,000 persons, and PDAC was the fourth-most frequent cause of death among all cancer diseases (www.svod.cz).

Using a variety of screening techniques, which included high-resolution melting (HRM) analysis, Sanger sequencing of whole genes, and next-generation sequencing (NGS), with a CZECANCA panel that was generated in our laboratory, we analysed several different cohorts of unselected PDAC patients and non-cancer controls. A role of PALB2 as a PDAC-predisposing gene in the Czech population was confirmed with the frequency of truncation mutations 1.97% (3/152) among unselected PDAC patients and 0.08% (1/1,226) in controls [odds ratio (OR)=24.66; 95% confidence interval (CI) 2.55-238.64; p=0.005]. Further, the recurrent Slavic mutation c.657_661del in *NBN* was found to have a higher frequency among unselected PDAC patients (5/241; 2.08%) than in controls (2/915; OR=9.65; 95% CI 1.9-50.2; p=0.006) and may represent another allele associated with an increased risk of PDAC occurrence. Moreover, NGS sequencing performed on 113 unselected PDAC patients revealed the presence of mutations in PDAC/HBOC (hereditary breast and/or ovarian cancer) predisposition genes in 15 patients [*BRCA1* (3x); *BRCA2* (5x); *PALB2* (1x); *ATM* (1x); *NBN* (1x); *CHEK2* (3x); and *BRIP1* (1x)]. The most promising new identified candidate gene was *LIG4,* which displayed a similar frequency of pathogenic variants as *BRCA1* and *CHEK2* (3x). In a unique group of 20 patients who survived more than 5 years after PDAC diagnosis, we identified five mutations in four genes, among four patients (4/20; 20%): *ATM* (1x), *MLH1* (1x), *RAD51D* (1x), and *CHEK2* (2x).

A set of missense *PALB2* sequence alterations, which were classified as variants of uncertain significance, were functionally characterised, *in vitro,* using a human U2 OS cell line as a model. The

analysed sequence variants were first introduced into the genome using clustered regularly interspaced short palindromic repeat (CRISPR)-based homology-directed repair. The primary PALB2 function within the DNA-damage response pathway during homologous recombination was determined in selected clones that harboured the analysed sequence alterations. The results showed no significant changes in homologous recombination activity among heterozygous clones with c.82_83delinsGC and c.3494C>T mutations, compared with the wild-type control.